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Molecular modeling of singlet-oxygen binding to anthraquinones in relation to the peroxidating activity of antitumor anthraquinone drugs*

Adam Liwo[a], Danuta Jeziorek[b], Tadeusz Ossowski[a], Dariusz Dyl[b], Anna Tempczyk[c], Jolanta Tarasiuk[d], Małgorzata Nowacka[a], Edward Borowski[d] and Wiesław Woźnicki[b]

[a]*Department of Chemistry, University of Gdańsk, J. Sobieskiego 18, 80–952 Gdańsk, Poland,*
[b]*Institute of Physics, Nicholas Copernicus University, Grudziądzka 5, 87–100 Toruń, Poland,*
[c]*Agouron Pharmaceuticals Inc., 11099 North Torrey Pines Rd., La Jolla, CA 92037, U.S.A.,*
[d]*Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, G. Narutowicza 11/12 , 80–952 Gdańsk, Poland*

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Anthraquinone derivatives are important anti-cancer drugs possessing, however, undesirable peroxidating and, in consequence, cardiotoxic properties. This results from the mediation by these compounds of the one-electron reduction processes of the oxygen molecule, which produces the highly toxic superoxide anion radical and other active oxygen species. This article summarizes the results of our studies on the molecular aspects of the mechanism of anthraquinone-mediated peroxidation which were carried out using enzymatic-assay, electrochemical, and quantum-mechanical methods.

Among the antitumor drugs the anthracycline antibiotics such as daunorubicin and doxorubicin [1] (Fig. 1) are some of the most effective DNA-intercalating agents [1, 2] However, the use of anthracycline drugs in clinical treatment is severely limited by their high cardiotoxicity [3–9]. The essential mechanism of this cardiotoxicity is related to their peroxidating activity, as a result of mediation by these compounds of the generation of superoxide anion radicals and other active oxygen species in the process of one-electron transfer from NAD(P)H to molecular oxygen [3–11]. These species are highly toxic, mainly with regard to their effect on the unsaturated lipid components of the cell membrane [12, 13] which, such as cardiolipin, are in abundance in the pericardium. The heart tissues possess a low level of superoxide dismutase and other superoxide-neutralizing enzymes and are, therefore, particularly susceptible to superoxide toxicity.

It has been generally accepted that the first stage of the electron-transfer mediation by anthraquinones involves one-electron reduction

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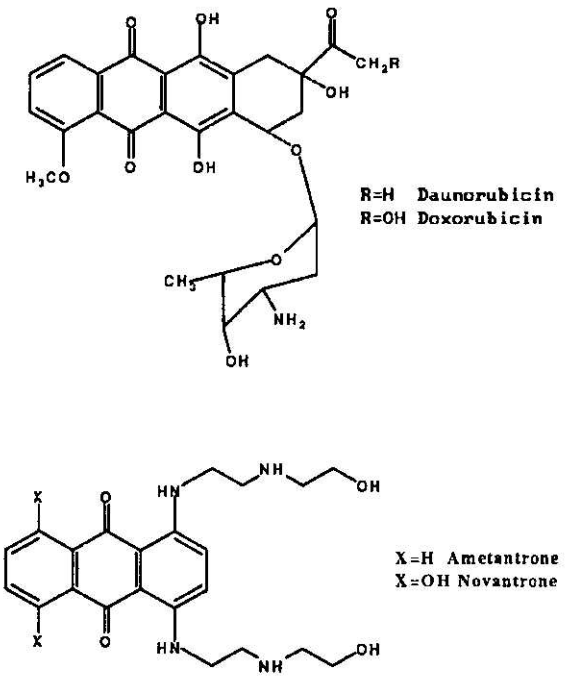


Fig. 1. Molecular structures of some representative anthracenedione-derived anticancer drugs.

of anthraquinone to semiquinone at the ubiquinone-reducing site of NADH dehydrogenase (complex I) present in cardiac mitochondria [7, 8, 14, 15]. One-electron reduction to semiquinone also occurs at the microsomal cytochrome P-450 reductase [16] and microsomal NADH cytochrome-b_5 reductase [17]. The semiquinone anion radicals could subsequently interact with molecular oxygen, transferring the excess electron to it with the formation of the superoxide anion radical. This route will hereafter be referred to as mechanism I [7, 8]. The presence of semiquinone radicals upon incubation in anaerobic conditions of heart and liver mitochondrial preparations containing NADH dehydrogenase and cytochrome P-450 reductase, respectively, was evidenced by ESR spectroscopy [4, 8]. No detailed molecular aspects of mechanism I were, however, proposed.

Based on the results of cyclic-voltammetry studies and semiempirical CNDO/2 calculations [18], we postulated that the mechanism of electron transfer may differ. In the first step, binding of the singlet ($^1\Delta_g$) oxygen by anthraquinone can occur, the formed complexes can then undergo one-electron reduction and finally dissociate into superoxide and original anthraquinone. This route will hereafter be referred to as mechanism II. The above hypothesis was supported by the fact that oxygen interaction with anthraquinones was manifested in cyclic-voltammetry experiments on daunorubicin by appearance of a new peak at a potential more positive than that of daunorubicin [18]. This peak gradually disappeared in deoxygenated solutions. Non-peroxidating anthraquinones, such as ametantrone, did not exhibit any additional peaks in cyclic-voltammetry experiments. Furthermore, semiempirical CNDO/2 calculations showed that the equilibrium distance of the oxygen molecule from daunorubicin is only 1.4 Å, which is characteristic of covalent interactions. The interaction of molecular oxygen with anthracyclines [19] or their iron(III) complexes [20, 21] was shown to occur in oxygenated aqueous solutions of these compounds on visible-light irradiation, to give ultimately the superoxide anion radical and products of anthracycline oxidation. Singlet-oxygen addition to unsaturated and also aromatic compounds is well known in organic chemistry [22]. On the other hand, the influence of oxygen on the process of daunorubicin reduction can also be explained in terms of interaction of oxygen molecules with semiquinone; therefore mechanism I cannot be ruled out.

We have carried out extensive investigation on the subject by means of cyclic-voltammetry [18, 23], enzymatic assays [23], and theoretical methods [18, 23–26]. The results of these studies are summarized in the present article.

FURTHER EXPERIMENTAL EVIDENCE FOR STRONG INTERACTION BETWEEN ANTHRAQUINONE DERIVATIVES AND MOLECULAR OXYGEN DURING THE REDUCTION PROCESS

Following the study of daunorubicin and ametantrone [18], we carried out a cyclic-voltammetry study of a series of model anthraquinones with different peroxidating activities, measured as the *in vitro* NADH oxidation rate [23]. The structures of these compounds, and their NADH oxidation rates, are shown in Fig. 2. We found that new reduction peaks appear mostly for anthraquinones with a high NADH oxidation rate. We also noted that new peaks appear in alkaline solutions (pH = 10) for all anthraquinones with phenolic groups (which should be deprotonated at this pH). This is

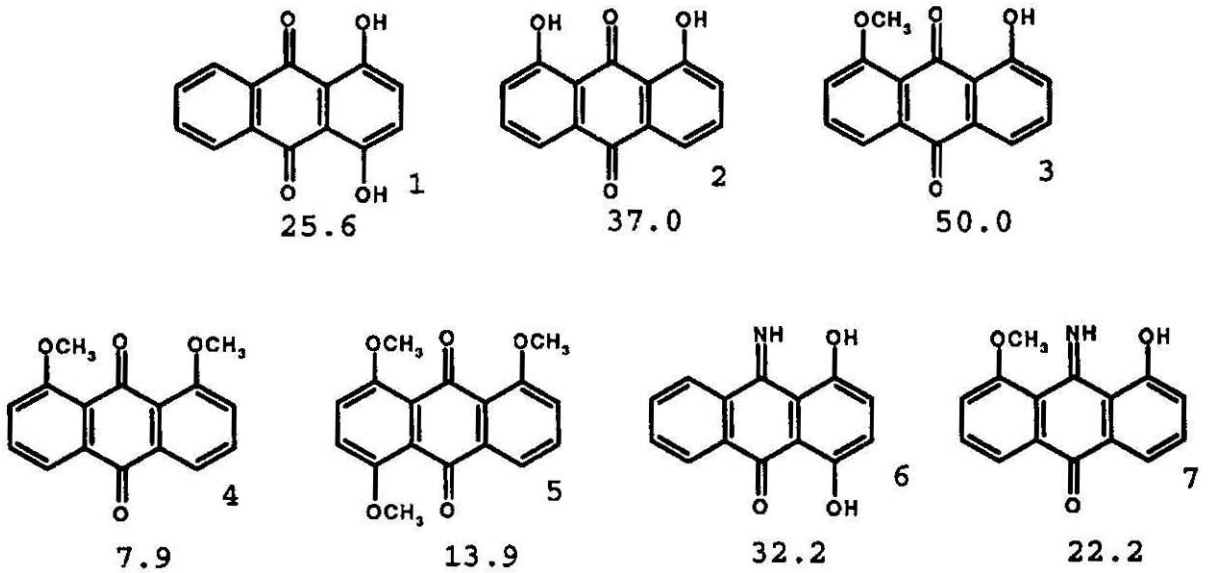


Fig. 2. Molecular structures of model anthraquinones used in enzymatic-assay and electrochemical studies. The numbers are the rates of NADH oxidation stimulated by these compounds (data from [23]).

consistent with the hypothesis of oxygen-adduct formation, because such a process involves partial charge transfer to the attached oxygen molecule, which will require a lower energy in the case of a negative anion of the base conjugated to hydroxyanthraquinone [18, 23–25].

The above-mentioned cyclic-voltammetry experiments were carried out in aqueous solutions in which oxygen reduction to superoxide takes place at a less negative potential (–0.33 V for neutral and alkaline pH [27]), as compared with those for reduction of anthraquinones to their semianthraquinones (of the order of –0.6 V [23]). This is due to efficient solvation of the small negative superoxide ion by water molecules [27]. Therefore the new peaks in cyclic voltammograms observed for some anthraquinone derivatives could be caused by the interaction of superoxide anion radicals with anthraquinones, and not by the interaction of anthraquinones or semianthraquinone anion radicals with molecular oxygen. Moreover, owing to proton-exchange phenomena, the superoxide ion is in equilibrium with the $HOO^{\bullet}$ radical ($pK_a = 4.69$) which can react with it to form hydrogen peroxide and oxygen [27, 28]. All these species have very high redox capacity and can interact with anthraquinone before it is reduced. In contrast to this, in aprotic solvents, such as dimethylsulfoxide (DMSO), the superoxide anions are solvated far less efficiently than in water and, in consequence, the reduction potential of oxygen is significantly more negative [27], with a value of –0.86 V [29]. Therefore, we carried out a cyclic-voltammetry study of some model anthraquinones considered in a previous paper [23] using DMSO as a solvent (Jeziorek, D., Ossowski, T., Liwo, A., Dyl, D., Nowacka, M. & Woźnicki, W., unpublished.). The results, summarized in Table 1, show that the influence of oxygen on anthraquinone reduction can be exhibited in two ways: either the anthraquinone-reduction peak is shifted towards more positive potentials (i.e. the reduction is facilitated) or, for 1-hydroxy-8-methoxy-9-iminoanthracene-10-one (7, Fig. 2), a new peak appears. The anthraquinone-reduction peaks also became gradually irreversible with increased oxygen concentration, which may indicate further rearrangement of the reduced oxygen adducts.

Note that for anthraquinones that do not bear any proton-donor groups in the outer rings the reduction process is not influenced by the presence of oxygen. This point will be addressed in below.

In conclusion, the results of cyclic-voltammetry experiments provide evidence for strong interaction between molecular oxygen and anthraquinones or their anion radicals. It is therefore reasonable to assume that covalent oxygen adducts are formed as intermediates in the electron-transfer process.

Table 1

Effect of oxygen on one-electron anthraquinone reduction in DMSO solutions as measured by cyclic-voltammetry.[a]

See Fig. 2 for compound designation. E^1_{pc} and E^1_{pa} denote the potential (V) of reduction (cathode) and back-oxidation (anode), respectively, *irv* or *ads* in place of anode-peak potential indicate that the back-oxidation peak vanished (lack or reversibility) or adsorption peaks appeared instead, respectively. The concentrations of anthraquinones were 8.0×10^{-4} M, oxygen concentration in oxygen-saturated solutions was 2.2×10^{-3} M.

System	Anthraquinone		Oxygen		Other	
	E^1_{pc}	E^1_{pa}	E^1_{pc}	E^1_{pa}	E^1_{pc}	E^1_{pa}
O_2			–0.870	–0.795		
1	–0.625	–0.565				
1 + O_2	–0.500	*irv*	–0.830	–0.735		
2	–0.630	–0.565				–0.580
2 + O_2	–0.460	*irv*	–0.860	–0.780		
3	–0.765	–0.700				
3 + O_2	–0.734	*irv*	–0.837	–0.622		
4	–1.050	–0.978				
4 + O_2	–1.050	–0.975	–0.850	–0.780		
5	–1.145	–1.080				
5 + O_2	–1.130	–1.065	–0.840	–0.775		
7	–0.945	–0.870				
7 + O_2	–0.9	*ads*	–0.830	*ads*	–0.590	–0.360

[a]Jeziorek, D., Ossowski, T., Liwo, A., Dyl, D., Nowacka, M. & Woźnicki, W., unpublished.

POSSIBLE MECHANISMS OF ELECTRON-TRANSFER MEDIATION

The initial phase of quinone/semiquinone-oxygen interaction should involve the formation of a non-covalent complex A...O_2 or $A^{\bullet-}$...O_2 (A = anthraquinone), which can then rearrange to form a covalent intermediate. The driving force of this process is provided by the interaction between the permanent dipole of the anthraquinone molecule or the negative charge of its semiquinone anion radical, and the induced dipole of the polarizable oxygen molecule [18]. For anthraquinones without a proton-donor group the possible covalent intermediates are peroxides, while for anthraquinones bearing proton-donor groups, oxygen binding can be accompanied by proton transfer to the attached oxygen molecule to form hydroperoxides or their anion radicals with a general stoichiometry AOOH or $AOOH^{\bullet-}$, respectively. The formation of hydroperoxides was well evidenced on photooxygenation of unsaturated and aromatic compounds bearing hydroxy groups [30–34], while peroxides are formed on photooxygenation of unsaturated [35–38] and aromatic [39] compounds. Regardless of the form of the intermediate, the next stage in the case of mechanism II should involve its one-electron reduction to form $AO_2^{\bullet-}$ or $AOOH^{\bullet-}$ and, finally, for both mechanisms I and II, the dissociation of the reduced complex into anthraquinone and superoxide (with possible formation of a non-covalent anthraquinone--superoxide complex A...$O_2^{\bullet-}$. This is illustrated in Fig. 3.

The basic difference between mechanisms I and II is that the oxygen molecule must be in the singlet $^1\Delta_g$ state in order to get attached to a neutral (i.e. non-reduced) anthraquinone derivative molecule (stage IIb), while both singlet and triplet $^3\Sigma_g^-$ oxygen can interact with semiquinone. Singlet oxygen required for this process is known to be produced in a variety of mitochondrial processes [13], and by photosensitized anthracyclines [40] and other sensibilizers [41], in the following sequence of photoexcitation and interconversion reactions:

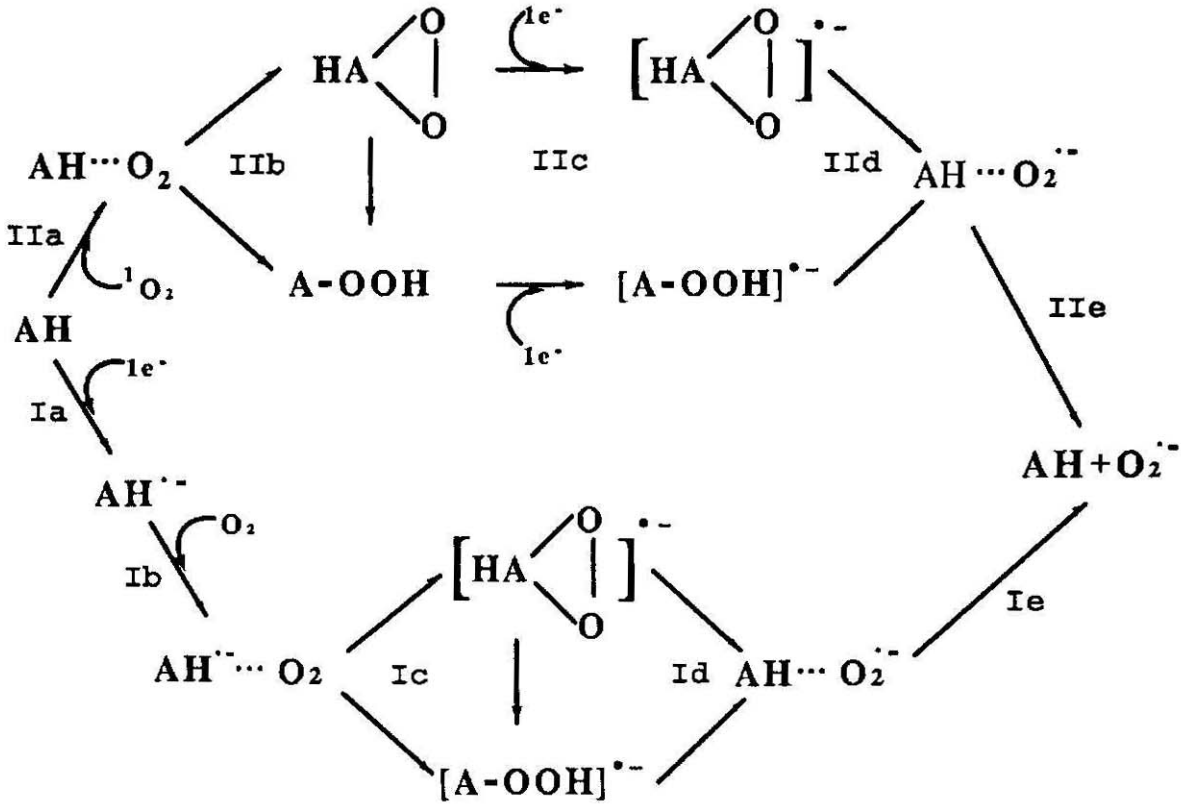


Fig. 3. Possible routes of anthraquinone-mediated electron transfer to molecular oxygen.

$$^1A \xrightarrow{h\nu} {}^3A^*$$

$$^3A^* + {}^3O_2 \rightarrow {}^1A + {}^1O_2^*$$

where ^1A and $^3A^*$ denote the sensibilizer molecule in the ground singlet state and excited triplet state, respectively.

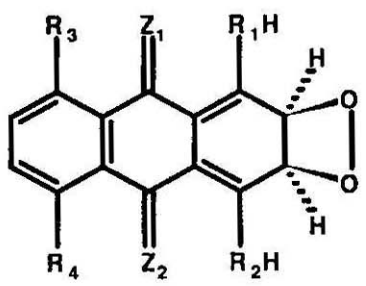
THEORETICAL STUDIES OF THE ENERGETICS OF VARIOUS STAGES OF THE ELECTRON-TRANSFER PROCESS

The molecular structures of possible intermediates of anthracenedione involved in the electron-transfer process are depicted in Fig. 4.

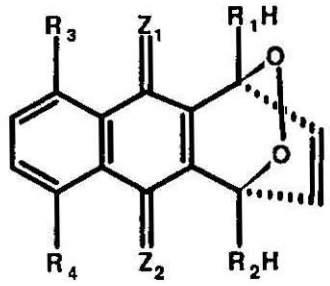
In a series of extensive quantum-mechanical calculations, with the use of *ab initio* and semiempirical methods [24–26], we have evaluated the energetics of formation and reduction of peroxides and hydroperoxides of various types.[1]

The simplest models to study oxygen binding are 1,4-dihydroxy- and 1,4-diaminobenzene, which simulate the outer rings of the anthraquinone moiety of the anthracycline and ametantrone series, respectively (Fig. 1). Table 2 summarizes the results of *ab initio* and semiempirical calculations of the energetics of formation of oxygen adducts to these compounds. As shown, hydroperoxides are clearly favored energetically over peroxides in the case of 1,4-dihydroxybenzene. For 1,4-diaminobenzene, hydroperoxide formation is less favorable, owing to a higher deprotonation energy of the amino, as compared to the hydroxy group. Note also that the semiempirical PM3 method overestimates the enthalpy of formation of peroxides relative to that of hydroperoxides, as compared with the results of RHF 4-31G and 6-31G *ab initio* calculations; the minimal STO-3G basis set gives an even lower hydroperoxide formation energy compared to those of the peroxides. Bearing in mind that hydroperoxides, and not peroxides, are the products of photooxygenation of phenols [32–34] and naphthols [31], the results of 4-31G and 6-31G *ab initio*

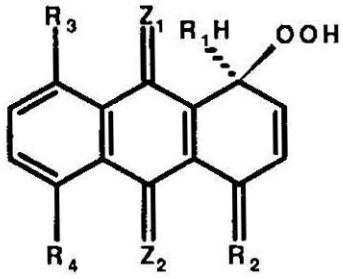
[1]The software used for the *ab initio* calculations was the MOTECC [42] and GAMESS [43] packages, while MOPAC [44, 45] was used for semiempirical calculations.



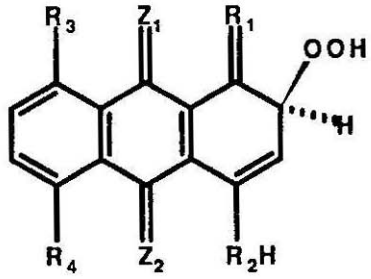
2,3-peroxide



1,4-peroxide



1-hydroperoxide



2-hydroperoxide

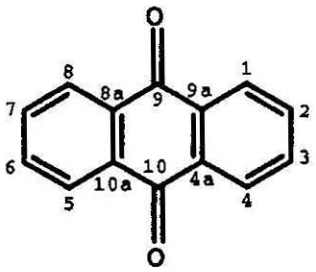


Fig. 4. Molecular structures of low-energy oxygen adducts to anthracenediones bearing proton-donor groups. Z_1, Z_2 = O or NH; R_1, R_2 = O or NH. The atom-numbering system of the anthraquinone moiety is in the bottom part of the figure. The depicted structures are only representatives of the families of structures that can be created by attaching the (hydro)peroxide moiety to all equivalent carbon atoms of anthraquinone; e.g. the 1-hydroperoxide can denote the 1-, 4-, 5- or 8-hydroperoxide.

calculations should be considered more reliable. Another argument for the adequate quality of the *ab initio* calculations is that they give the geometry of the peroxy and hydroperoxy moieties consistent with available experimental data; the semiempirical PM3 method gives excessive O–O and C–O bond lengths [24–26] (other modern semiempirical methods available in the MOPAC package, MNDO and AM1, give even greater discrepancy between the calculated and experimental geometries [24, 25]).

To study the energetics of reduction of anthraquinones and their peroxides or hydroperoxides, or of the binding of oxygen to semiquinones, it is not sufficient to consider outer benzene rings of the anthraquinone moiety, because the excess electron is transferred mainly to the central quinone part. Therefore the simplest model compound is 1-hydroxynaphtoquinone; it contains both the reducible quinone moiety and an aromatic ring with a hydroxy group that permits formation of both peroxides and hydroperoxides. However, this limits the level of *ab initio* calculations to the 4-31G basis set (the minimal STO-3G basis set cannot be used, because the results are too divergent from those of higher basis sets). The results of calcu-

Table 2

Energies (ab initio) and enthalpies (PM3) of oxygen addition to 1,4-dihydroxybenzene and 1,4-diaminobenzene involving the formation of the 2,3-peroxides and hydroperoxides calculated by various theoretical methods (kcal/mol) [a]

Species	1,4-Dihydroxybenzene				1,4-Diaminobenzene			
	STO	4-31G	6-31G	PM3	STO	4-31G	6-31G	PM3
2,3-O_2	–22.8	9.4	12.1	–1.6	–26.5	3.7	6.7	–3.4
1,4-O_2 [b]	–16.4	17.1	17.5	–1.3				
1-OOH	–11.1	–25.7	–23.2	–16.6	–2.8	–10.4	–7.7	–1.2
2-OOH	–9.4	–16.8	–14.1	–18.3	–3.1	–8.0	–5.6	–9.0

[a]Data from [24] and [26]; the energies of peroxide and hydroperoxide formation were recalculated to be expressed relative to the oxygen $^1\Delta_g$ singlet state.

[b]For 1,4-diaminobenzene calculations were not carried out on this peroxide.

lations of the energetics of reduction of 1-hydroxynaphtoquinone and its (hydro)peroxides (stage Ia of mechanism I and stage IIc of mechanism II), oxygen binding to the neutral compound (stages IIa and IIb of mechanism II) and semiquinone (stages Ib and Ic of mechanism I), as well as the total energy of reductive oxygen binding to 1-hydroxynaphtoquinone (stages Ia-Id and IIa-IId of mechanisms I and II), are summarized in Table 3 (Jeziorek, D., Ossowski, T., Liwo, A., Dyl, D., Nowacka, M. & Woźnicki, W., unpublished).

As in the case of 1,4-dihydroxybenzene, the hydroperoxides again have a higher energy of formation than peroxides. Even more important is the fact that the reduction energies of hydroperoxides are about 20 kcal/mol lower than the reduction heats of the parent compound; therefore oxygen addition leading to hydroperoxide formation will greatly facilitate the reduction process, if electron transfer is governed by mechanism II. The 1,4-peroxide also has a higher reduction energy than the parent compound, but its low formation energy makes it a less likely intermediate than the hydroperoxides.

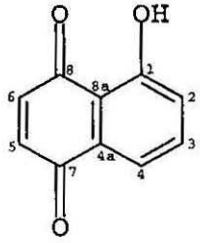


Fig. 5. Numbering of atoms in 1-hydroxynaphtoquinone.

Table 3

Calculated energies (ab initio) and enthalpies (PM3) of oxygen addition to 1-hydroxynaphtoquinone ($\Delta E_{IIa+IIb}$), reduction of 1-hydroxynaphtoquinone and its peroxides and hydroperoxides (ΔE_{red}), oxygen addition to semiquinone (ΔE_{Ib+Ic}), and total energies of (hydro)peroxide anion radical formation (ΔE_{sum}) [a, b]

Species	$\Delta E_{IIa+IIb}$		ΔE_{red}		ΔE_{Ib+Ic}		ΔE_{sum}	
	4-31G	PM3	4-31G	PM3	4-31G	PM3	4-31G	PM3
Q			–21.9	–49.6				
2,3-O_2	8.3	–4.2	–21.0	–46.9	9.1	–1.5	–12.3	–51.2
1,4-O_2	12.3	2.1	–39.9	–63.1	–5.7	–11.3	–27.6	–61.0
2-OOH	–0.2	–8.2	–44.7	–59.7	–23.0	–18.3	–44.9	–67.9
4-OOH	–3.6	–1.8	–42.1	–54.9	–23.9	–7.1	–45.9	–56.8

[a]Jeziorek, D., Ossowski, T., Liwo, A., Dyl, D., Nowacka, M. & Woźnicki, W., unpublished.

[b]See Fig. 5 for atom numbering.

It should be noted at this point that the energies of hydroperoxide formation ($\Delta E_{IIa+IIb}$) are by more than 10 kcal/mol less than those of 1,4-dihydroxybenzene and, in fact, the 4-31G estimated values are close to zero. This results from increasing the ionization potential when condensing a (poly)hydroxybenzene with electron-withdrawing quinone moiety, because oxygen addition involves charge transfer from anthraquinone to oxygen and thus depends on the electron-donating capacity of the anthraquinone molecule [26]. Therefore the hydroperoxides of anthraquinones should be considered as possible intermediates in the electron transfer process, rather than stable compounds that could be isolated. In fact, to our best knowledge, they have never been isolated on photooxidation of anthraquinone derivatives. Even the hydroperoxides of hydroxybenzenes and hydroxynaphtalenes which, according to the results of our calculations, should be much more stable thermodynamically, have a half-life time at room temperature usually shorter than one hour [32–34] and then rearrange to give more stable products of oxidation.

If the reduction process is governed by mechanism I, the energy of oxygen addition to semiquinone is a quantity of interest. As shown, this energy is also greatest if the hydroperoxide anion radical is an intermediate. This finding also remains valid when considering the total energy change of the processes leading to the reduced (hydro)peroxides; in this case it is not important whether electron-transfer mediation occurs *via* mechanism I or mechanism II. Therefore, according to the results of energy calculations, hydroperoxides and/or their anion radicals, rather than peroxides, should be considered as the most probable intermediates in the electron-transfer process. This conclusion is in excellent agreement with the results of cyclic-voltammetry experiments described in the preceding section: anthraquinones without hydroxy groups underwent electrochemical reduction independent of the presence of oxygen, while for anthraquinones bearing the hydroxy groups either the position of the anthraquinone reduction peak was shifted towards more positive potentials or a new peak appeared at a more positive potential [18, 23]. The shift of the peaks towards more positive potentials also is consistent with the fact that the calculated energy of reductive oxygen addition to 1-hydroxynaphtoquinone, with formation of reduced hydroperoxides, is greater than the energy of the reduction of the parent compound or oxygen (Table 3).

The 4-31G estimated energy for dissociation of the 4-hydroperoxide of 1-hydroxynaphtoquinone anion radical (the intermediate with the lowest energy) into superoxide and naphtoquinone amounts to +24.8 kcal/mol (Jeziorek, D., Ossowski, T., Liwo, A., Dyl, D., Nowacka, M. & Woźnicki, W., unpublished), so this process is unfavorable energetically and should not occur readily *in vacuo*. Bearing in mind that superoxide is thermodynamically stabilized in water relative to aprotic solvents, we calculated the energy of dissociation of a *protonated* form of the 4-hydroperoxide anion radical into 1-hydroxynaphtoquinone and the $HOO^{\bullet}$ (hydroperoxy) radical. In this case, the energy change is –6.7 kcal/mol, which favors dissociation. Thus, the final stage of superoxide release might involve the diffusion of the hydroperoxide anion radical from the largely nonpolar ubiquinone-reducing site of NADH dehydrogenase [46] to the aqueous inter- or intracellular solution, where it can undergo protonation, or at least form hydrogen bonds with water protons, and dissociate into anthraquinone and the hydroperoxy radical.

RELATIONSHIP BETWEEN THE CALCULATED ENERGIES OF (HYDRO)PEROXIDE ANION RADICAL FORMATION AND PEROXIDATING ACTIVITY OF ANTHRAQUINONES

If covalent interaction occurs between molecular oxygen and anthraquinone or semiquinone during the electron-transfer process, one can expect some correlation between the peroxidating properties and the energy of oxygen binding. The most relevant quantity in this regard is the total energy of reductive oxygen binding to form $AO_2^{\bullet-}$ or $AOOH^{\bullet-}$, because it depends on the overall energetics and not on the specific pathway for its formation (mechanism I or II). In our last paper [26], we performed semiempirical PM3 calculations of the energetics of oxygen addition and reduction of a series of model anthraquinones, whose peroxidating ability had been measured earlier as the rate of NADH oxidation [23] (see Fig. 2 for structures).

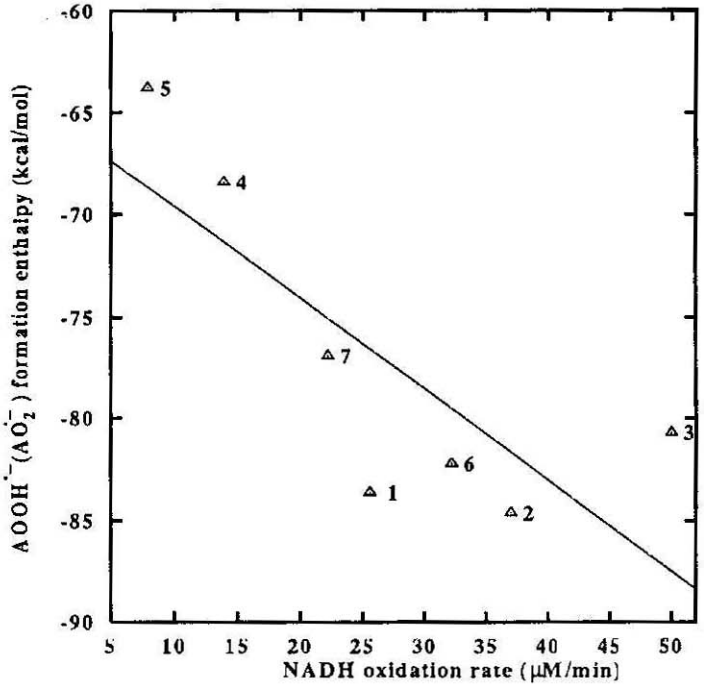


Fig. 6. Correlation of NADH oxidation rate (data from [23]) and the PM3-calculated enthalpy of reductive oxygen addition to anthraquinones to form a peroxide or hydroperoxide anion radical (Jeziorek, D., Ossowski, T., Liwo, A., Dyl, D., Nowacka, M. & Woźnicki, W., unpublished). The equation is V_{max} *= −0.50- (0.19) ΔH −58.2(5.6) with the correlation coefficient of 0.7695.*

The use of *ab initio* methods is, unfortunately, prohibited by the size and number of compounds to be examined. All these compounds are at least fairly good substrates of NADH dehydrogenase, so it is reasonable to assume that the differences in their peroxidating activity are caused mainly by different redox properties and not different affinity for the enzyme. The correlation between NADH oxidation rate and the energy of (hydro)peroxide anion radical formation is shown in Fig. 6 (for anthraquinones bearing hydroxy groups, the compounds considered were hydroperoxides; and for anthraquinones without such groups, peroxides). As shown, the more negative the enthalpy of oxygen binding, the greater the NADH oxidation rate. The correlation coefficient is 0.7695 (59% of explained variance). This finding is a further argument for the mediation of covalent oxygen adducts to anthraquinones or semiquinones during the electron-transfer process. Similar correlations occur between the NADH stimulation rate and the enthalpies of oxygen binding to anthraquinones or semiquinones [26].

Because semiempirical PM3 calculations are relatively inexpensive, the existence of the above-mentioned correlations provides a rapid means for the estimation of the peroxidating properties of anthraquinones.

CONCLUSIONS

The results of our studies on electron-transfer mediation to molecular oxygen by anthraquinone derivatives have shown that electron transfer is likely to involve the formation of hydroperoxide or peroxide anion radicals. They can be formed either by one-electron reduction of anthraquinone to semiquinone followed by oxygen addition (route I), or by singlet-oxygen addition to the neutral anthraquinone molecule, followed by one-electron reduction (route II). Superoxide can be released as the $HOO^{\bullet}$ radical after protonation of the hydroperoxide anion radical. Owing to a comparatively high ionization potential of anthraquinone derivatives, their oxygen adducts should have considerably diminished formation energies compared with those of phenols and naphtols which form metastable hydroperoxides [32–34]. Therefore, they can only be considered as short-living intermediates in the electron-transfer process. In fact, in contrast to phenols and naphtols, oxygen adducts have never been isolated on photooxygenation of anthraquinones.

Since, according to results of theoretical calculations, the formation of hydroxperoxide anion

radicals results in about twice as much energy gain, compared to anthraquinone reduction alone, their formation explains the shift of anthraquinone-reduction potentials towards lower values. The formation of hydroperoxides is less favorable energetically in the case of amino derivatives, owing to a greater energy of proton abstraction from the amino, compared to the hydroxy group. For anthraquinones without proton-donor groups, only peroxides can be formed which, however, have both formation and reduction energies remarkably lower than hydroperoxides. These findings are consistent with the remarkably diminished peroxidating activity of the amino (e.g. ametantrone) [9] and methoxy [23] derivatives of anthraquinones compared to hydroxyanthraquinones.

From the above considerations it can be concluded that the ability of anthraquinone derivatives to mediate electron transfer to molecular oxygen results from the presence of both reducing (benzene rings with hydroxy groups) and oxidizing (quinone moiety) sites in one molecule, so the molecule can both trap oxygen and undergo one-electron reduction. Therefore the molecular determinants of electron-transfer mediation should comprise both redox (ionization potential and electron affinity) and acidic-basic properties (which are important in hydroperoxide formation).

Because we investigated only the energetics of (reductive) oxygen binding to anthraquinones, we cannot draw any conclusion, at the moment, as to which mechanism, I or II, actually governs the electron-transfer-mediation process. To draw such conclusions, calculations should be carried out the entire reaction path, including the transition states. We have already made some progress in *ab initio* studies of oxygen binding to unsaturated alcohols, the simplest models of the oxygen-binding site of anthraquinone derivatives.

It should be noted that peroxidating properties of anthraquinones are of a very complex nature and the ability to bind oxygen is not their only determinant. Affinity for NADH dehydrogenase (substrate properties) also is essential and was found to depend strongly on anthraquinone structure [47]. Nevertheless, the results of our present studies allow for a better understanding of the molecular origin of the peroxidating activity of anthraquinone-derived anti-cancer drugs, and could be of value for design of new drugs devoid of the undesirable peroxidating and, thus, cardiotoxic properties.

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